New Investigation Tools to Predict Percutaneous Penetration

Elsa Jungman, Cécile Laugel, Spiro Khoury, Arlette Baillet-Guffroy
Groupe de Chimie Analytique Paris Sud (EA4041) Equipe de Lipides Cutanés
School of Pharmacy Paris-Sud 11, 5 rue JB Clément, 92290 Châtenay Malabry, France
Correspondance: elsa.jungman@gmail.com

Objective

The stratum corneum plays a barrier against exogenous molecules penetration due to its lipidic matrix composed of three major lipids: ceramides, fatty acids and cholesterol. Nowadays, in accordance to the OECD recommendations, percutaneous penetration prediction is based on molecules structural characteristics i.e. Log P (polarity) and Mw (molecular weight). Our work consisted of developing an additive parameter to examine ceramide-molecule interaction. The experimental design included various cosmetic molecules i.e. Log P ranging between -0,07 and 6,88 and Mw between 152 and 361. We studied their cutaneous dictribution with Franz cells coupled to HPLC analysis. In parallel we followed molecules skin distribution and stratum corneum lipids status by FTIR microspectroscopy (synchrotron source). Ceramide-molecule interaction was studied by affinity chromatography. This investigation gives new tools to modulate percutaneous penetration prediction.

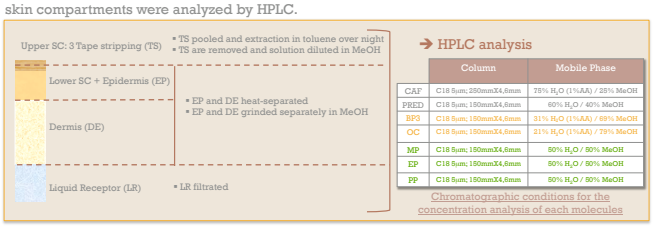
Molecules		Properties	MW	Log P
Caffeine	CAF	Cellulite reduction	196	-0,07
Prednisolone	PRE	Corticosteroid	360	1,62
Benzophenone 3	BP3	UV filter	228	3,79
Octocrylene	OC	UV filter	361	6,88
Methyl Paraben	MP	Preservative	152	1,93
Propyl Paraben	EP	Preservative	166	2,27
Ethyl Paraben	PP	Preservative	180	2,81

Molecules chosen for the study according to their physico-chemical properties

Franz cells Experiments coupled to HPLC analysis

• Method

Skin penetration studies were performed with a Franz cells device. Fatty tissue from fresh human abdominal skin was removed. 200µl of the solution (2 µmol of the molecule dissolved in ethyl acetate) was dropped on the skin surface. The experiment was realized in triplicate. After 22 hours, the skin surface was washed and the stratum corneum (SC) was tape-stripped 3 times. All skin compartments were analyzed by HPLC.

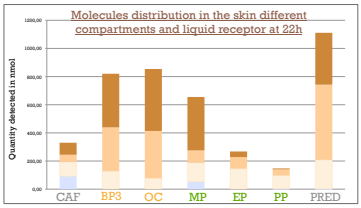


➔ HPLC analysis

	Column	Mobile Phase
CAF	C18 5µm; 250mmX4,6mm	75% H_2O (1%AA) / 25% MeOH
PRED	C18 5µm; 150mmX4,6mm	60% H_2O / 40% MeOH
BP3	C18 5µm; 150mmX4,6mm	31% H_2O (1%AA) / 69% MeOH
OC	C18 5µm; 150mmX4,6mm	21% H_2O (1%AA) / 79% MeOH
MP	C18 5µm; 150mmX4,6mm	50% H_2O / 50% MeOH
EP	C18 5µm; 150mmX4,6mm	50% H_2O / 50% MeOH
PP	C18 5µm; 150mmX4,6mm	50% H_2O / 50% MeOH

Chromatographic conditions for the concentration analysis of each molecules

• Results



	TS	EP	DE	LR
CAF	85±6	51±14	102±32	91±62
PRED	369±148	534±99	207±91	1±2
BP3	381±30	311±74	128±82	0±0
OC	440±173	336±36	77±27	0±0
MP	380±40	88±12	135±35	52±8
EP	40±40	82±43	146±70	0±0
PP	5±3	46±29	94±94	2±3

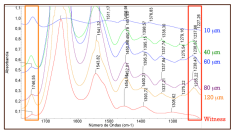
Molecules quantity (nmol) in the skin different compartments and liquid receptor

➔ These Franz cells datas will serve as reference for the FTIR microspectroscopy and the affinity chromatography.

Experimental Approach: FTIR Microspectroscopy

• Method

Biopsies were removed at 22h from franz cells and frozen at –20 °C, -80 °C. Biopsies were cryotomed at -20°C and mounted on CaF_2 lames. FTIR microspectroscopy was performed on Synchrotron (SMIS beam-line at *SOLEIL*). Spectra were recorded at a resolution of 4 cm^{-1} between 4000 cm^{-1} and 800 cm^{-1} or 650 cm^{-1} with 32 accumulations and a pixel size of 6 µm x 6 µm. A skin with no molecules was also tested to serve as blank.

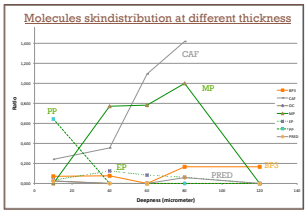


Caffeine spectra at different deepness and in the blank skin

Spectra Analysis

The lipidic barrier status (νCH_2) was observed between 2849-2854 cm^{-1}. Molecules distribution was examined at 10 µm (stratum corneum); 40, 60, 80, 120 µm (epidermis).
A specific band, that was not observed in the blank skin, was selected for each molecule spectra. The reference band was recorded at 1745 cm^{-1}.
The ratio *specific band / reference band* was calculated for each deepness to draw the distribution profile of each molecule.

• Results



	νCH_2
CAF	2854
PRED	2851
BP3	2854
OC	2851
MP	2851
EP	2855
PP	2854
BLANK	2848

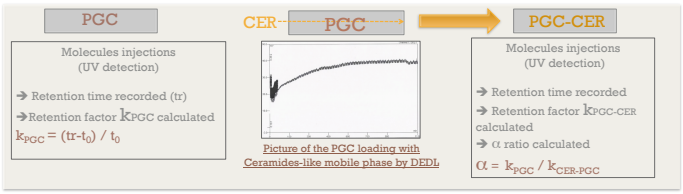
$\nu CH2$ of the stratum corneum lipids in cm^{-1}

➔ FTIR microspectroscopy shows an illustration of the molecule skin distribution and helps to follow the biopsy lipidic status. The molecule distribution within the skin is a qualitative information. It is difficult to quantify our results. The distribution here shows the molecule detection at a precise deepness in the epidermis and doesn't represent skin compartments as it is the case in the Franz cells experiments (EP, DE etc..).

Predictive Approach: Affinity Chromatography

• Method

We developped a method by affinity chromatography to mimic Ceramide-Molecule interaction in the stratum corneum. A Porous Graphit Column (PGC) was modified with a mobile phase containing ceramides-like molecules. The PGC loading was followed with a DEDL. Before and after the loading the molecules of interest were injected and detected by UV. Retention times were recorded and compared.



• Results

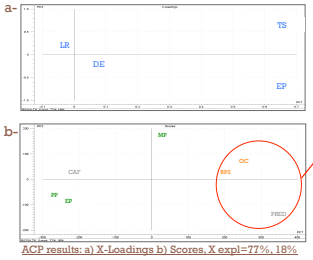
	k_{PGC}	$k_{PGC-CER}$	α
CAF	26,5	19,9	1,3
PRED	7,7	5,4	1,4
BP3	26,3	18,3	1,4
OC	4,2	3,1	1,4
MP	6,1	3,4	1,8
EP	6,3	3,7	1,7
PP	8,4	5,0	1,7

Retention factors and ratio calculated

➔ Molecules chromatographic behavior involves the interaction between the ceramide and the molecule analyzed.
➔ The α ratio is a complementary parameter to Log P and MW.

Results treatment by Chemometrics

It was not possible to perform a Linear Multiple Regression analysis (LMR) due to a poor molecules number. We performed instead a principal component analysis (ACP) in order to highlight variability sources of the percutaneous penetration profiles of the molecules studied in Franz cells.



ACP results: a) X-Loadings b) Scores, X expl=77%, 18%

	α
CAF	1,3
PRED	1,4
BP3	1,4
OC	1,4
MP	1,8
EP	1,7
PP	1,7

α ratio determined by affinity chromatography

➔ The ACP shows that in the Franz cells experiments, CAF, PP and EP tend to be more present in the dermis and liquid receptor whereas UV filters (BP3 and OC) and PRED tend to be more retained in the stratum corneum and epidermis. Especially for PRED that is a good epidermis representation.
➔ PRED, BP3 and OC have the same ratio α value. It shows that these 3 molecules may have a similar interaction with ceramides in the stratum corneum.

Conclusion & Perspectives

To Predict percutaneous penetration Log P and MW are not enough relevant parameters. That is why our studied introduced an additive parameter that focused on ceramides-molecules interaction. This investigation was a feasibility study that provided new trends and next these experiments are going to be reproduced with a much higher molecules number in order to realize PLS. In parallel we are developing a new method to investigate ceramide-molecule interaction by fluorescence.
The FTIR microscopy datas highlight vibrational methods to develop in vivo investigation (ex: Raman)